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MORPHOMETRICAL INVESTIGATIONS ON THE REPRODUCTIVE ACTIVITY OF THE OVARIES IN RATS SUBJECTED TO IMMOBILIZATION AND TO MOTION ACTIVITY

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16. Abstract

Wistar-strain white female rats divided into three groups, first group subjected to motion loading, second used as control, the third group was immobilized. A considerable reduction in numbers of corpora lutea was observed in the immobilized group, together with small number of embryos, high percent of embryo mortality, fetal growth retardation, and endometrium disorders. Control group showed no deviation from normal conditions, and there was slight improvement in reproductive activity of animals under motion loading.

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MORPHOMETRICAL INVESTIGATIONS ON THE REPRODUCTIVE ACTIVITY OF THE OVARIES IN RATS SUBJECTED TO IMMOBILIZATION AND TO MOTION ACTIVITY

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One of the current problems in contemporary biology is the problem of increased and decreased motion activity on the organism. Over the last two decades, interest in researches along these lines have significantly increased as a result of the progressive decrease in muscle loading in contemporary man, and because of attempts to more rapidly concur outer space. In connection with this, the question of reproductive feasibilities in the female organism placed under conditions of hypodynamia or hyperdynamia is especially important. This question has a direct connection with animal protein production in the cultivation of agricultural animals because of the prolonged periods of time that they are subjected to conditions of hypokinesia. /66*

In a small number of communications, investigations have taken place regarding the influences on enhanced or lowered functional loading with physical work on sexual behavior, the structure and functioning of the ovaries, duration of gravidity, natality rates, and the growth and development of offspring and their viability.

Micue [18] has established that forced, strenuous running in mice for up to six days after breeding resulted in a blocking effect on gravidity, either preventing implantation of the fertilized cell, or cutting short an early gravidity. Moderate physical loading up to 13 days of gravidity, however, leads to the birth of offspring with better developed skeletons in comparison with mice taken from animal controls.

*Numbers in the right margin indicate pagination in the foreign text.

Fatyushin and co-authors [5] have elucidated the role of various loadings, differing in degree, on the organism accompanied by physical work in the development of morphological changes in the ovaries of rats. With moderate loading, there were no divergences from norms determined. An increase in the loading provoked an intensification in blood supply to the ovaries and an increase in the number of follicles and corresponding corpora lutea. Intensified muscular activity, however, led to an atresia on the follicles or to their systic degeneration, and in some cases this reached the stage of hyperplasia on the stroma ovarii. /67

Mateeff, et al. [13, 14] found that higher natality rates are generated in families by training the male and female rats, and the lowest natality rates are in families formed of immobilized animals. The authors note, in addition, that motion loading does not prevent a full-term gravidity and the birth of vital offspring.

Stroganova [4] has demonstrated that under the effects of a 62-day period of immobility, the sexual behavior of female rats was altered; this was expressed in shows of aggression towards the male mice during the first days of cohabitation. This led to a delay in the births by two to three days in comparison with the control group. The progeny of the immobilized mothers were significantly slowed in their developments during the first two weeks.

Euker, et al. [7] determined with female mice that as a result of stress induced by three days of immobility before mating or by one to five days of immobility after gravidity, in one case birth was given in term with a normal number of offspring in one litter, and in the other case, the embryos perished.

There are also studies demonstrating what kinds of changes take place in the estrous cycle of female animals under the effects of increased or decreased locomotive activity.

Svechnikova and co-authors [3] established that moderate physical

loading caused only insignificant changes in the overall duration of the estrous cycle in female rats, as well as on individual phases of the cycle. Enhanced motion activity, however, led to significant changes in the organism's hormonal balance, as a result of which the quietus phases and the estrous phases were prolonged. Strenuous loading with physical work suppressed ovary functioning to a significant degree.

Pokhlenchuk and co-authors [2], although they used different models for physical exercise, established that in female rats locomotive activity does not change the overall duration of the estrous cycle, and it changed the duration of the individual phases in only 60% of the test animals. In the remaining portion, the estrous phase was prolonged in 20% of the rats, and in the other 20% it was the diestrous phase that was prolonged.

We have posed the problem of confirming whether or not any changes in ovary function comes about in rats that are subjected to an immobilization regime and to those subjected to motion activity. For this purpose, we have employed the following indices: functional activity of the gonads in unfecundated animals, together with their potentional reproductivity, and embryo reproductivity and embryo mortality in those that were fecundated.

THE STUDY PROPER

Material and Methods

The experiment was begun with 90 female rats of the Wistar strain with initial ages of 45 days, divided equally into three groups: physiologically immobilized, a control group, and those with motion loading. All animals were subjected to normal variations in the conditions, that is, temperature, humidity, illumination, and ventilation, and they all received water and food at will.

The immobilized rats lived continuously in individual cages for

physiological immobilization, original design (Penchev, et al., 1969). With our immobilization method, locomotor activity is reduced to a minimum, but it is possible to move for feeding, drinking water, and /68 for grooming (Fig. 1).

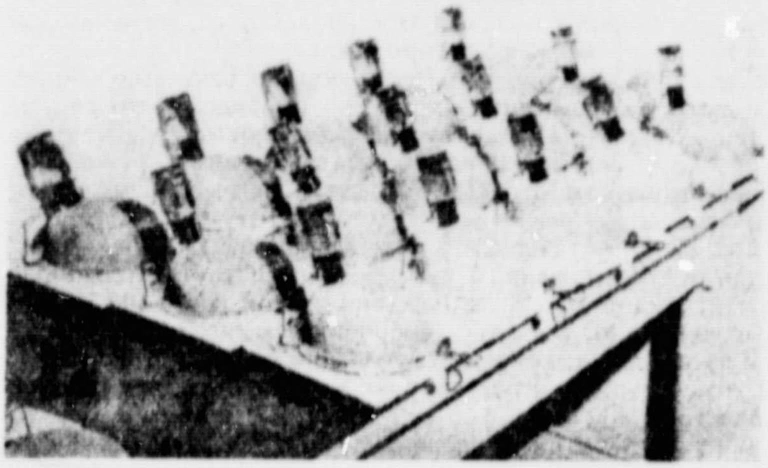


Fig. 1

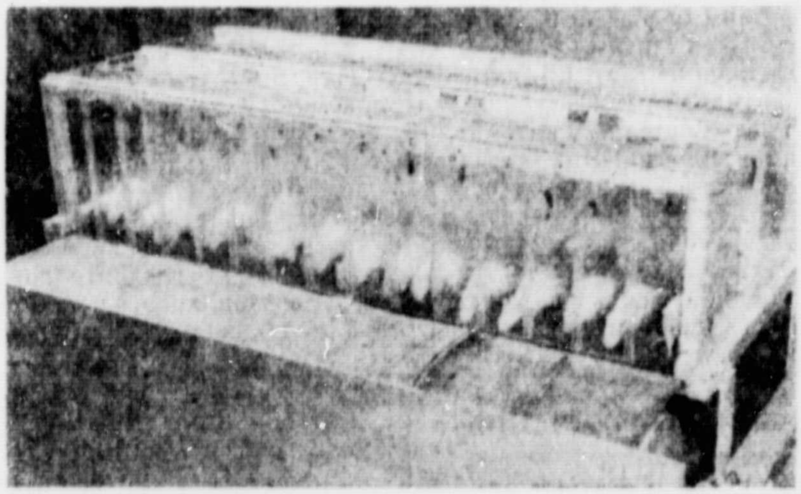


Fig. 2

The control animals were maintained in groups of five in standard, plastic cages.

The rats subjected to motion loading were exercised for six days per week on the tretbahn according to Kimeldorf with three velocities, I — 550, II — 703, and III — 940 m/h. During the test period, they covered in all 242,043 m, of which 36,461 m were at velocity I, 166,787

covered in all 242,043 m, of which 36,481 m were at velocity I, 166,787 at velocity II, and 38,775 m at velocity III. These lived also in five per cage, as with the control animals (Fig. 2).

After 580 days of immobilization for the first group, and 500 days respectively for the control group, and 490 days of exercise for the rats undergoing motion loading, several days before the term of gravidity, all animals were sacrificed by means of decapitation. /69

We opened the abdominal cavity and the uterine tubes in order to check for the presence and number of embryos, the number of clearly perished fetuses (according to the remains of the placenta), and the number of partially reabsorbed or mummified fetuses in the animals that had been fecundated. The ovaries on all the rats were placed in a 4% neutral solution of formalin, and after 48 hours, using a magnifying lens, we counted the number of corpora lutea and the corresponding number of ovulations.

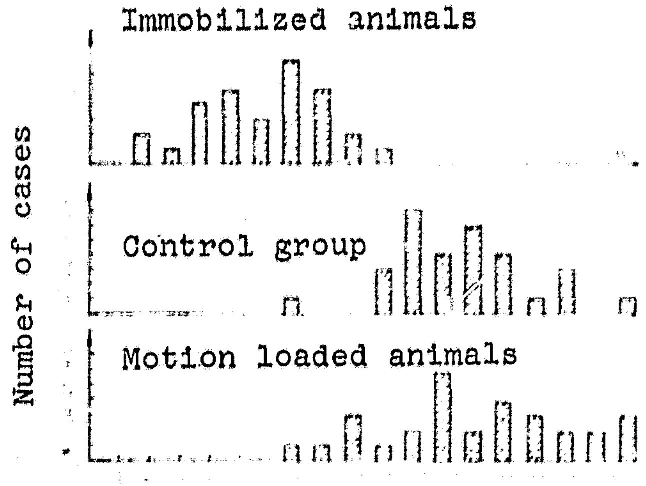
The data derived in this manner were processed by means of variational statistical means with a determination of the individual indicators: the mean arithmetical value, the mean error for the mean arithmetical value, reliability for the mean error, correlation coefficient, and with a determination of the mean error for these two coefficients.

Results

The results from the data derived for the three groups of animals are given together with corresponding numerical values for all indicators and are illustrated with graphs.

The functional activity of the female gonads and the unfecundated rats were counted according to the number of ovulations and the corresponding number of corpora lutea in the two ovaries. We determined 183 corpora lutea in the immobilized group (average 6.1 ± 0.44), in the control group 377 (average 12.57 ± 0.49), and in the group that was motion loaded 393 corpora lutea (average 13.1 ± 0.31). From the numerical data

and their average values, it may be seen that the differences between the two experimental groups are quite large. The results are statistically reliable between the immobilized and control group, and the immobilized and the motion loaded group at p=0.001. In Fig. 3, a visual representation of the number of corpora lutea in all the unfecundated animals is given, according to the number of cases. In the immobilized



Overall number of corpora lutea
Fig. 3

group, there is a strong impression of a displacement towards the left, towards the smaller number of corpora lutea. In the other two groups, there is a significant displacement to the right. In the motion loaded animals, this is especially characteristic, and in contrast with the /70
control group animals, they have an even distribution of instances with numbers of corpora lutea from 7 to 18. It is only in this group that cases with 17 and 18 corpora lutea are well expressed in the unfecundated animals.

In the same way as for the first index, we determined the potential reproductivity in the fecundated rats according to the number of corpora lutea in the ovaries. In the immobilized animals, we found 293 corpora lutea (average 9.77±0.42), 332 in the control group (average 11.7±0.54), and with the motion loaded animals 368 corpora lutea (average 12.27±0.44). These data show the same tendencies to a differentiation in the number of corpora lutea between the three groups, as in the calculations for the index of functional activity of the ovaries. Here,

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as well, the differences between the immobilized and the control animals and between the immobilized and the motion loaded animals are significant at p=0.001. These results are represented in Fig. 4. It is

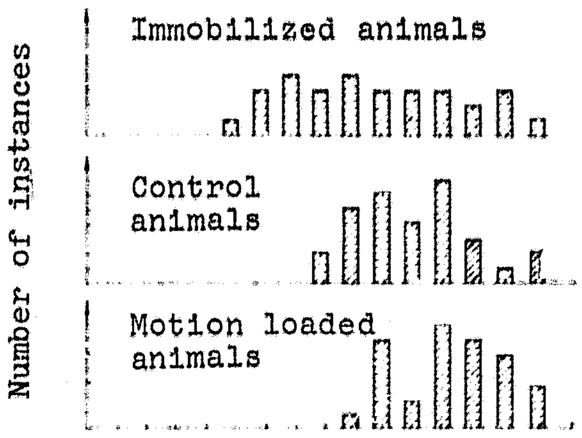
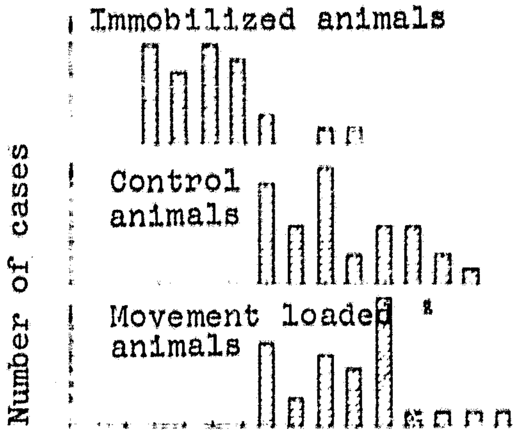


Fig. 4

constructed on the basis of the overall number of corpora lutea in the two ovaries of all the fecundated animals and the number of instances. It may be seen that in the motion stressed animals there is a better expressed shift to the right, because in contrast to the control group and especially in contrast to the immobilized group, the numbers of cases with 13, 14, and 15 corpora lutea predominate.

We calculated embryo reproductivity according to the number of embryos in the uterine tube for each fecundated rat. In the immobilized group, this was 136 (average 4.53±0.35), in the control group 281 (average 9.37±0.42), and 296 in the motion loaded group (average 9.81±0.34). There is a strong impression here of a significant difference in the number of embryos in the two experimental groups, which is pronounced in favor of the group of motion loaded animals. This means that in a state of immobilization, only 46.42% of ovulated ova were fecundated and developed fetuses, while in the group of animals undergoing systematic muscle loading, this percent increases to 80.43%. In the computation of this index as well, the results are significant between the immobilized group and the control group and between the immobilized animals and the movement loaded animals at p=0.001. These results are

clearly shown in Fig. 5. Fig. 5 is constructed on the basis of the overall number of embryos and the number of cases. The strong displacement to the left, toward the smaller number of fetuses, is very /71 clearly expressed in the immobilized rats.



Overall number of embryos

Fig. 5

With the movement loaded animals, there is a good impression that the difference between the control group is expressed in the large number of instances of 11 embryos, while with the immobilized animals not one female animal is found with more than 10 fetuses. Characteristic as well for the animals subjected to movement loading is the fact that it is only in that group that we found cases where there were 15 embryos present.

In calculating the index of embryo mortality, we determined that in the immobilized group in all 157 ovulated and fecundated ova and embryos perished (average 5.23±0.41). In the other two groups, these values were significantly lower, that is, respectively 50 (average 1.67±0.62) for the control group, and 78 (average 2.6±0.31) for the animals undergoing movement loading. These results are statistically reliable only with the immobilized animals and the control animals and between the immobilized animals and the movement loaded animals.

Fig. 6 shows the number of perished fecundated ova and embryos in /72

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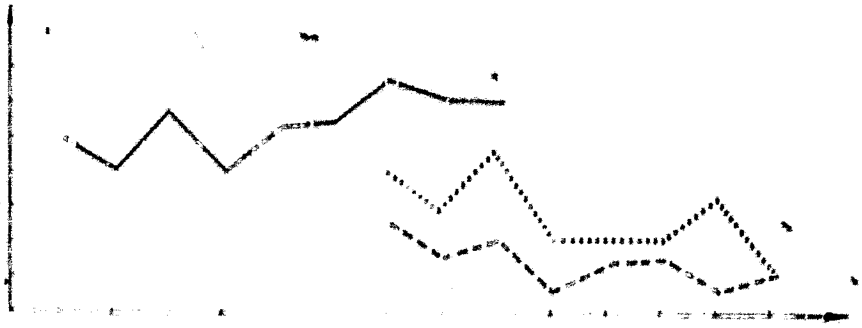
the three groups of animals, as well as in relation to the overall number of embryos. Especially well expressed here is the early embryo



Overall number of embryos

Fig. 6. 1 — immobilized; 2 — movement loaded; 3 — control

mortality in the immobilized rats. Despite the fact that the number of embryos in this group is the smallest, their mortality is quite significant and sharply differentiated from the mortalities in the other two groups.

From Fig. 4, it may be seen that the curves for the control animals and the movement loaded animals are in agreement from the onset of gravidity to the twelfth day. Differences in embryo mortality between these two show up in the last days with a smaller number of instances in favor of the control animals.

Discussion

There are reports and communications in the literature which elucidate to a considerable degree the influences of motion activity and hypokinesia on the development of morphological and functional changes in the system of sex-related phenomena (Fedorov and co-authors [6], Lamb, et al. [12], Mateev, et al. [15], Palazhchenko [1]). There are also data demonstrating the effects of certain exogenous stimuli on the female sex organs (Jöchle [9], Whitten [19], Grönroos, et al. [8], Stegmann, et al. [17]). In this connection, our studies are also concerned with the duration of the estrous cycle and its specific phases

in rats under conditions of hypodynamia and hyperdynamia (Konstantinov, et al. [10]). Under the influence of motion loading, we have determined a prolongation of the active phases, that is, estrus and metestrus, while with immobilized animals, the phases of quietus increase, that is, diestrus and partly proestrus. Our former data are in direct relation to the results of our present investigation. In calculating the indicators or indices for the functional activity of the ovaries in unfecundated females and the potential reproductivity in gravid females, we had anticipated an increase in the number of ovulations and corresponding number of corpora lutea in groups undergoing motion loading, and corresponding decreases in these values in groups of immobilized animals in relation to control animals.

The results from the present investigation show, as well, that differences in the motion activity regimes have a direct relationship to one of the basic problems in contemporary biology, that is, the question of reproductive feasibilities of the organism placed under normal and extreme conditions. To a great extent, this depends both on the implantation of the fecundated ovum, as well as on the growth, development, or perishing of the germ cell. The increased number of embryos with animals subjected to motion loading and the increased number of perished fecundated ova and germ cells in the immobilized animals demonstrates that under the influence of immobilization, the reproductive capacity of an organism is reduced due to the increase in its embryo mortality. These results find their explanation in another of our works, by means of which we elucidated the influence of muscular loading and hypokinesia on morphological and histochemical changes in rat ovaries (Konstantinov, et al. [11]). The facts that we established there, namely atresia on the follicles, hypoplasia and atrophy of the ovarian tissue, the presence of ovarian cysts, an increase in their cholesterol and neutral fats, the decrease in the content of polysaccharides and phospholipids in immobilized animals, all show the reduction in the functioning of the female gonads as a result of this immobilization. Those results supplement and fully support the results from this experiment; this demonstrates that in contrast to systematically applied muscular loading, constant immobilization leads to a reduction in

ovarian functional activity, to reductions in potential and embryo reproductivity, whereas at the same time giving rise to an increase in early embryo mortality.

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